INTERLEUKIN-6 AS A PREDICTOR FOR PEDIATRIC TRAUMATIC BRAIN INJURY RECOVERY

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In partial fulfillment of the requirements for graduation with Health Science Scholars Honors Degree in Neuroscience

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1 Abstract

Traumatic brain injury (TBI) is a leading cause of death and disability around the world, making it a major public health concern. The post-injury condition is marked by a complex, pathophysiological nature and wide-ranging physical, emotional, and cognitive symptoms. We hope to explore the connection, if any, between interleukin-6 (IL-6) levels and patient recovery following a head injury in pediatric patients. Essentially, we predict that higher IL-6 levels would correspond with more unfavorable patient outcomes following injury, and this relationship would be found to be more robust in TBI participants. To forward this objective, we used serum IL-6 samples and three surveys completed at baseline, 3 months, and 6 months since the initial hospital visit. We compared these values to a set of pediatric orthopedic controls to isolate TBI-specific biomarker-outcome interactions. Thus far, our findings indicate that IL-6 levels are a poor predictor for patient post-injury outcome for TBI cases and a moderately good predictor in orthopedic controls. Yet, on average, participants who reported worse outcomes had higher IL-6 levels, even across both groups. These findings allow for us to understand how certain biomarkers, such as IL-6, may play a role in predicting TBI-specific recovery in pediatric patients.

2 Introduction

Traumatic brain injury (TBI) is an acute cranial condition that is often marked by loss of consciousness and/or alteration of mental status due to blunt, physical damage and/or piercing entry into brain tissue (Morita, et.al. 2017). Afflicting all demographic groups, an estimated 288,000 hospitalizations and 56,800 deaths will be related to TBI annually (Center for Disease Control and Prevention, 2019). Despite this, the complex pathophysiological nature of cranial trauma presents a clinical challenge to understand the chronic and sometimes progressive

symptoms that manifest unequally among affected patients (Vassar, et. al. 2014). In this sense, a more robust comprehension of its symptoms, underlying causes, and eventual outcomes is necessary to determine the proper course of action for proper prognosis, drug development, and psychosocial treatment.

Despite decades of clinical study, the body of research surrounding TBIs have disproportionately surrounded the neuropathology of adult TBI rather than their pediatric counterparts (Figaji, 2017). Due to these age-related differences in brain development, post-injury consequences may reveal dissimilarities with the pathophysiology of adult TBI compared to pediatric TBIs (Madikians, et al. 2007). Proper diagnosis and therapy that are restricted to the specific pathological condition of a pediatric patient is greatly needed. It is paramount to discern the variation among pediatric TBI victims against the adult population in order to accomplish more targeted care.

When subject to physical stresses, neuro-inflammatory mechanisms are put into motion that are primarily supported through the intracranial production of interleukins, other cytokines, and various additional inflammatory proteins. Used to stimulate blood flow and remove damaged tissues, the process by which interleukin-6 (IL-6) promotes inflammation involves the activation of various regenerative systems and the recruitment of additional protein and cellular factors that aid in the restorative management of disease or damage (Rose-John et. al., 2011). In this case, IL-6 offers itself as an ideal candidate to measure relative extent of inflammation in a patient and the aftereffects observed following a TBI or an orthopedic injury.

Self-reported health surveys present valid means to accurately account various psychological and physical states at a given time (Segerstrom et. al., 2016). Combined with IL-6 levels taken from pediatric patients, self-reported health outcomes are crucial to understand any

potential psychiatric comorbidities that may be expressed following the distressing experience of a TBI. Taken together, these measures assess the interplay between IL-6 concentration, current psychosomatic conditions, and future predictors of prognosis and health consequences.

In this present study, serum IL-6 levels and self-rated health measures were taken from both TBI-related pediatric cases and pediatric orthopedic injury controls. With the aim of understanding the differences in IL-6 levels predicting overall health outcome between TBI cases and orthopedic controls, our overarching objective was to understand the particular role of IL-6 that is primarily in TBI rather than a more generic bodily injury. As well, we hope to report any discernible differences in IL-6 levels that are specific to certain demographic groups and mechanism of injury.

3 Methods

3.1 Study Design Overview

This study is a prospective, observational study in humans. We approached and enrolled participants ranging from age five to sixteen with either head or orthopedic injuries for the cases and control group, respectively. Blood tube collections and multiple parent proxy and pediatric surveys were completed in order to enroll in the study. Blood was then processed and stored in the form of serum to measure IL-6 concentrations using a single-plex immunoassay instrument, SMCxPRO (Millipore Sigma), that uses a sandwich-based ELISA method.

3.2 Participant Enrollment

3.2.1 Inclusion and Exclusion Criteria

Participant enrollment required the fulfillment of certain inclusion and exclusion criteria. The following inclusion were: (1) the participant must be within five to sixteen years of age at the time of enrollment, (2) the visit reason must be indicative of possible head injury, brain injury, concussion for TBI cases and possible fracture, sprain, contusion, or unspecified bodily trauma for orthopedic controls, and (3) the injury must have had to occur within the past twenty-four hours prior to enrollment. Exclusion criteria included: (1) previous diagnosis of any significant cranial or orthopedic injuries, (2) current or recent use of anti-inflammatory medicines or treatments, and (3) major developmental or chronic disorders such as microcephaly, severe cognitive impairment, diabetes, and any inflammatory conditions such as juvenile arthritis.

3.2.2 Consenting Process

Following authorization by the International Review Board, potential participants were enrolled in the emergency department at Dell Children's Medical Center in Austin, Texas. All approaches were conducted by undergraduate and health professional volunteers who had completed the requisite training and background checks to enroll all cases and controls. Once agreeing to join the study, the participant is asked to fill out parental proxy and pediatric surveys through a secure portal and prepare for a blood draw performed by a registered nurse, licensed phlebotomist, or patient care technician.

3.3 Survey Collection

Surveys were generated by Patient Reported Outcome Measurement Information System (PROMIS) and Neurological Quality of Life (NeuroQoL) surveys, a National Institute of Health-developed and validated tool, which focuses on eight topics. These consist of five PROMIS surveys completed by both a parent proxy and pediatric participants that included depressive symptoms, anxiety, psychological stress, positive affect, and global health. Three NeuroQoL surveys were administered and completed by the pediatric participant that consisted of fatigue, cognitive function and social relations assessments. Surveys were between six to nine questions in length and took between five and ten minutes to complete each one. Copies of each survey are located in the *Appendix* section.

3.4 Biospecimen Processing and Storage

Blood will be collected into the blood collection tube and inverted at least 5 times, incubated upright for 30-45 minutes. Collected blood was placed in a biohazard bag for transport to the laboratory and processing.

After blood has been collected, the tube will be inverted 5-6 times and is left to stand in an upright position for 30-45 minutes to allow for clotting. Once evidence for clotting is observed, the tube will be centrifuged at 3500 RPM for 5 minutes. If the sample appears to be turbid, it will be centrifuged once more. Once turbidity is not present, the supernatant, or serum, is aspirated into 750 uL aliquots into individual cryovials using a transfer pipette.

3.5 IL-6 Measurement using ELISA

3.5.1 Standard Curve Preparation

This study prepared the standard curve in a 12-channel reagent reservoir. A 1:3 serial dilution was performed for standards 2 through 5 and 1:2 serial dilutions for standards 6 through 11 to achieve a curve from 50 pg/mL to 0.1 pg/mL with Standard 12 acting as the blank.

3.5.2 Plate 1 Capture, Prep, and Wash

Following the serial dilution, 75 µL of the standards were pipetted per well onto the assay plate. Following mixing of the coated beads, immediately before adding to the assay plate, this study added 550 µL of magnetic coated beads to 10.5 mL of supplied assay buffer. The bead vial was rinsed with 0.55 mL of assay buffer for complete bead transfer, and 100 µL of beads was aliquoted onto the assay plate. This plate was covered with clear adhesive plate seal, and incubated for 2 hours at 25°C on a microplate shaker at 875 RPM. Approximately 10 minutes prior to the end of target capture incubation, the detection antibody was prepared. Following incubation, the plate was centrifuged at 1,100 x g for 1 minute and washed with an automated plate washer.

3.5.3 Detecting, Washing, Shaking, and Aspirating

After removal from the plate washer, 20 µL per well of detection antibody was dispensed without disturbing the bead pellet. The assay plate was sealed with clear adhesive plate seal, incubated for 1 hour at 25°C on a microplate shaker at 1000 RPM, and washed four times on an automated plate washer.

After a four-cycle pre-transfer wash, the plate was placed on a microplate/incubator shaker for 90 seconds at 750 RPM. Once removed the plate shaker, the clear adhesive plate seal was carefully removed to avoid splashing and placed it on the plate washer to perform final aspiration of the sample.

3.5.4 Elution Buffer and Final Incubation

Next, we dispensed 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet, incubated plate for 10 minutes at 25°C on a microplate incubator/shaker at 1000 RPM. The plate was then incubated for 2 minutes prior to transfer to the 384-well plate.

A glass-bottomed 384-well Aurora plate was used for final transfer, in which 10 uL of Buffer D was added, followed by 10 uL of eluate from the first plate. This plate was covered with an aluminum seal and centrifuged at 1,100 x g for 5 minutes.

After the immunoassay instrument, SMCxPRO, equilibrated its internal temperature, the assay plate was placed inside the machine and analyzed, taking about 30 minutes until completion.

3.6 Statistical Analysis

All analyses were conducted using Microsoft Excel 2016 and R Studio. Independent sample t tests, Pearson regression analysis, and one-way ANOVA tests were conducted for all continuous variables. Serum IL-6 concentration, baseline, three month, and six month survey outcome parameters were presented as either number (percentage), mean (± SD), or median (interquartile range [IQR]), wherever appropriate. To be considered different, the group difference had to have a p value of < 0.05 on outcome variables. All t-scores from PROMIS and NeuroQoL

outcome surveys were generated through the HealthMeasures Scoring Tool, a national database that compares this study's outcome values with general and clinical reference populations. Percent changes were calculated as a relative comparison between one t-score with a previous t-score to measure shift in responses over time.

4 Results

4.1 Demographic Profile

A total of 68 participants (54.4% of them male) with a mean age 11.17 years, with an age range of 5 through 16 years were analyzed for the current study (Table 1). The average IL-6 concentration in the cohort, taken holistically, is 2.49 pg/uL. Head or orthopedic injuries were mostly evenly distributed among accidents (33.8%), fall (29.4%), and sports-related reasons (32.4%), with only 4.4% being admitted into the hospital due to assault. Hispanics comprised 47.1% of the cohort and 72.1% of the population were white.

4.2 Exclusions and Survey Profile

Out of the 74 participants who were currently enrolled in the study, four of them were excluded due to failing to complete a baseline survey and another two were excluded as extremely high outliers, for a final sample size of 68. For the 3-month surveys, 38 were finalized, with 26 surveys completed by cases and another 12 finished by controls. For the 6-month survey, a total of 28 surveys were completed, as 20 were completed by the cases and another 8 by the controls.

4.3 Outcome Stratified by IL-6 Concentrations

Given by a percent change in outcome, a more negative score indicates worse outcome and a higher positive score indicates a better outcome. When measuring case baseline to 3-month percent change in self-reported outcome, IL-6 concentrations were significant predictors of overall anxiety values ($p < 0.01$). While weakly significant, pediatric psychological stress and positive affect were relatively predictive by IL-6 concentrations ($r2 = 0.123$ / $p < 0.173$; $r2 = 0.122$ / $p < 0.433$). Likewise, a percent change between case pediatric baseline and 6 months was significant for pediatric positive affect outcomes that was reasonably robust in its predictive nature ($r2 = 0.153$ / $p < 0.021$).

As well, IL-6 concentrations are also a predictor in the parental global health surveys completed by the control group, as the percent change from baseline to 3 months shows a negative trend of -6.74%, $p < 0.002$). Also, IL-6 concentration was significant in determining the overall percent change of pediatric social relations and positive affect in the control group, showing a -10.73% and a -7.94% change from baseline to 3 months ($p < 0.001$, $p < 0.003$).

Lastly, the percent change from baseline to 6 months in the control group shows the most significant effects in the parental anxiety and pediatric psychological stress, with a -13.58% and a -6.71% decrease in overall wellbeing ($p < 0.012$, $p < 0.018$). During the same period, a percent change of -4.30% was also observed for the pediatric global health survey ($p < 0.002$).

4.4 IL-6 Concentrations Stratified by Age and Sex

Split into three subsets, child (5-9 years), preteen (10-12 years), and teen (13-16 years), there was no difference between the groups ($p < 0.501$) or when further stratified by cases and controls ($p < 0.103$, $p < 0.132$, $p < 0.193$).

No difference between male and females as a collective group ($p < 0.342$) or when separated based on cases or controls ($p < 0.324$, $p < 0.492$).

4.5 IL-6 Concentrations Stratified by Ethnicity and Race

As well, there were no differences between Hispanic and non-Hispanics groups when taken as a collective ($p < 0.425$) or when separated based on cases or controls ($p < 0.452$, $p < 0.104$). Likewise, using one-way ANOVA, there were no significant differences between races taken as a whole ($p < 0.121$), or when separated by cases or controls ($p < 0.183$, $p < 0.495$, $p < 0.249$).

4.6 IL-6 Concentrations Stratified by Mechanism of Injury

There was a significant difference in IL-6 levels when comparing the mechanism of injury as a total cohort, with those admitted into the hospital due to accidents having higher IL-6 concentrations (4.13 pg/uL) relative to other causes of injury. This difference is made more apparent when comparing the case and control groups together, as controls had increased levels of IL-6 compared to their counterparts (6.21 pg/uL, $p < 0.039$).

5 Discussion

5.1 Outcome Stratified by IL-6 Concentrations

As there were little relative percent change for the case pediatric baseline to 3-month psychological stress and positive affect surveys, -2.37% and 2.54% respectively, there was also a low degree of predictive power of IL-6 levels with its subsequent outcome. Here, with an r2 value of 0.123 and 0.122, respectively, it was noted that a higher concentration of serum IL-6 mildly

predicted a worse outcome for the pediatric participants documenting their own wellbeing (*Figure 1*). This is consistent with the background literature as IL-6 plays a significant role in determining overall welfare, as seen here by the pediatric self-reporting (Gill et. al. 2018). Most parental proxies and pediatric surveys, however, showed a negative percent change with relatively little predictive power, such as the percent change found in pediatric anxiety (*Figure 2*).

With a relatively large positive percent change for pediatric positive affect (11.25%) from baseline to 6 months, there was a significant relation between lower IL-6 levels and a better self-reported outcome ($p < 0.021$, *Figure 3*). As IL-6 levels increase, there is little or negative improvement in the participant's positive outlook. This is consistent with a lower baseline of IL-6 predicting a more robust, positive outcome (Wagner, et. al. 2014).

For the control group reporting between baseline and 3 months, there were significant percent changes self-reported by the pediatric participant, as depressive symptoms, psychological stress, and social relations had stark, negative percent changes (-5.29%, -7.94%, and -10.73%, respectively). These surveys had significant differences within the population that indicated similar trends with the control group ($p < 0.014$, $p < 0.003$, $p < 0.001$), yet lacked significant predictive power through the levels of IL-6 concentrations, with the exception of pediatric depressive symptoms (*Figure 4*). However, this finding is not consistent with the basis of higher IL-6 levels predicting a worse outcome, as this trend proved quite the opposite. This may have been due to sampling error as a relatively low sample size of 20 was used.

When comparing the relative percent change outcomes for control groups between baseline and 6 months, there were significant differences in the pediatric responses for psychological stress, global health, and cognitive function (-6.71%, -4.30% and 23.60%). Yet, much like the self-reports between baseline and 3 months, there was little predictive power between IL-6 concentrations and

outcome measures. As, pediatric social relation outcomes showed a high predictive strength between the serum biomarker and a more positive outcome. However, due to a very small sampling size of 8, any slight deviation from the normal proves to pull the r2 value away from the true value, which is evidenced by *Figure 5* and the relatively high p-value of 0.086, indicating no significant difference.

5.2 IL-6 Concentrations Stratified by Demographics

There were no significant differences found within any of the demographic groups when taken as a whole or when separated by cases or controls (Table 2). This shows that the relative distributions of IL-6 concentrations were equally measured across the demographic groups and had no impact on any of the outcome percent changes reported by the participants or their parental proxies. Overall, however, there was a significant difference in IL-6 levels between the case and control groups ($p < 0.018$, *Figure 6*).

5.3 IL-6 Concentrations Stratified by Mechanism of Injury

When measuring the relative concentrations of IL-6 across different mechanisms of injuries from both groups, there was a significant difference in those who were admitted into the hospital due to accidents ($p < 0.019$). As this group primarily consists of larger impact injuries, such as motor vehicle accidents, horse-related, and bicycle accidents, the trauma is expected to be greater and thus, have higher levels of inflammatory proteins, such as IL-6 and other interleukins and cytokines.

This difference is further held when comparing the case and control groups, as the control group had a much higher IL-6 concentration, on average, relative to the case group ($p < 0.039$) when stratified by mechanism of injury. With concentrations of 6.21 pg/uL and 2.70 pg/uL respectively, the control groups admitted to the hospital due to accidents had greater levels of IL-6 concentrations when compared to the case group, which may be indicative of the total-body nature of accidents attributed to large vehicle or animal accidents, contrasting local injuries affecting only the head and neck area, such as injuries relating to sports activity or falls.

5.4 Limitations

There were strong limitations within the study, with only 38 out of the 68 participants completing the 3-month survey and only 28 participants going on to complete their 6-month survey. This relatively low retention rate for survey responses proved to hinder the statistical strength of many of the responses and may have artificially raised the strength of others. Since the control group only had 8 participants with surveys completed after 6 months, any small differences between the values markedly skewed relative statistical significance, as 4 out of 13 surveys were deemed to be of noted difference and another 2 surveys were approaching significance. This lies in contrast to the 1 or 2 surveys presented to be of statistical significance in other time periods across the case and control groups, who had a more robust sampling size, buffering any potential distortion from individual values.

6 Conclusion

Overall, IL-6 concentrations were mild predictors in determining participant outcome over 3 month and 6-month periods. While certain outcomes had statistical significance and a strong

predictive attribute, such as pediatric positive affect from baseline to 6 months, most survey outcomes were weakly predicted by levels of IL-6 concentrations across both case and control groups.

However, many of the surveys had a robust percent change in both groups, with many of the changes having p-values below 0.05, across time periods. Many of the large percent changes, either positive or negative, were shown to have statistical significance across groups and showed how IL-6 concentrations may play a role in determining the relative outcome of participants enrolled in the study.

Demographics played little or no role in influencing serum IL-6 levels, either across both groups and when taken together as a whole. This allows for this study to tentatively conclude that the demographic profile of the participants had no impact on the relative outcome measured by the surveys. However, there was a significant difference between mechanisms of injury, as those who suffered accidents, such as vehicle crashes or bicycle incidents, were subject to much higher IL-6 levels in the control group. This finding may be attributed to more severe impacts related to total-body crashes often associated with moving at relatively high speeds,

Future work involves the use of genomic data to further elucidate the genetic difference in protein expression among individuals across the groups. This would allow future studies to control for single nucleotide polymorphisms that have an effect on overall IL-6 levels that may, in turn, govern pediatric and parent proxy outcome responses post-injury. As well, greater emphasis on patient retention in the form of survey responses is vital for the creation of a more cogent sample size. Moreover, greater research into other potential inflammatory proteins of interests, such as IL-10 and amyloid beta-42, would further distinguish different serum biomarkers that may play a role in outcome prognosis and change. Lastly, sex-age paired case-control would control for any

confounding differences to create a stronger study design to measure biomarkers and their role in predicting patient prognosis.

7 Acknowledgments

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9 Appendix

		Group		
	Total (N = 68)	TBI Case (n = 43)	Orthopedic Controls (n = 25)	*p*-value
Age (years, mean ± SD)	11.17 (2.92)	11.27 (2.87)	11.12 (3.05)	0.3461
Age Range (years)	(5 – 16)	(5 – 16)	(5 – 16)	
Sex, Male (%)	37 (54.4)	23 (53.4)	14 (53.8)	0.4539
Ethnicity, Hispanic (%)	32 (47.1)	20 (46.5)	13 (52)	0.4392
Race, White (%)	49 (72.1)	31 (72.1)	19 (73.1)	0.3291
Mechanism of Injury				
Accident (n, %)	23 (33.8)	15 (34.9)	8 (32)	0.3421
Assault (n, %)	4 (4.4)	4 (6.9)	0 (0)	-
Fall (n, %)	20 (29.4)	14 (32.6)	6 (24)	0.2428
Sports-Related (n, %)	22 (32.4)	11 (25.6)	6 (44)	0.2945

Table 1. Demographic Comparison between Cases and Controls. Significant *p*-values below < 0.05 are marked with an (*).

	Total (N = 68)	*p-value*	Group		
			TBI Case (n = 43)	Orthopedic Controls (n = 25)	*p*-value
Age Groups					
Age 5 – 9	3.19 (3.70)	0.501	2.52 (3.63)	4.34 (3.31)	0.103
Age 10 – 12	2.23 (2.39)		1.60 (1.17)	3.35 (4.49)	0.132
Age 13 – 16	2.21 (3.19)		1.48 (2.13)	3.43 (4.96)	0.193
Sex					
Male	2.07 (2.33)	0.342	1.51 (1.36)	3.07 (3.28)	0.324
Female	2.97 (3.62)		2.17 (2.76)	4.30 (4.54)	0.492
Ethnicity					
Hispanic	2.42 (2.92)	0.425	2.22 (2.81)	2.74 (3.18)	0.452
Non-Hispanic	2.56 (3.14)		1.46 (1.23)	4.08 (4.43)	0.104
Race					
White	2.77 (3.43)	0.121	1.97 (2.43)	4.17 (4.41)	0.183
Black or African American	1.63 (1.84)		1.14 (1.11)	2.63 (2.86)	0.495
Other	1.90 (0.73)		1.75 (0.81)	2.24 (0.91)	0.249
Mechanism of Injury					
Accident	4.13 (4.46)	0.019	2.70 (3.34)	6.21 (5.22)	0.039 **
Assault	1.63 (1.74)		1.63 (1.74)	-	-
Fall	1.79 (1.79)		1.39 (1.24)	2.97 (2.74)	0.143
Sports-Related	1.65 (1.27)		1.42 (1.02)	1.87 (1.49)	0.429
Overview (n, %)	2.49 (3.05)	-	1.82 (2.13)	3.66 (3.91)	0.018***

Table 2. IL-6 Concentration Between TBI Case and Orthopedic Control Groups. Values shown are (mean ± SD), with significant *p*-values below < 0.05 marked with an (*).

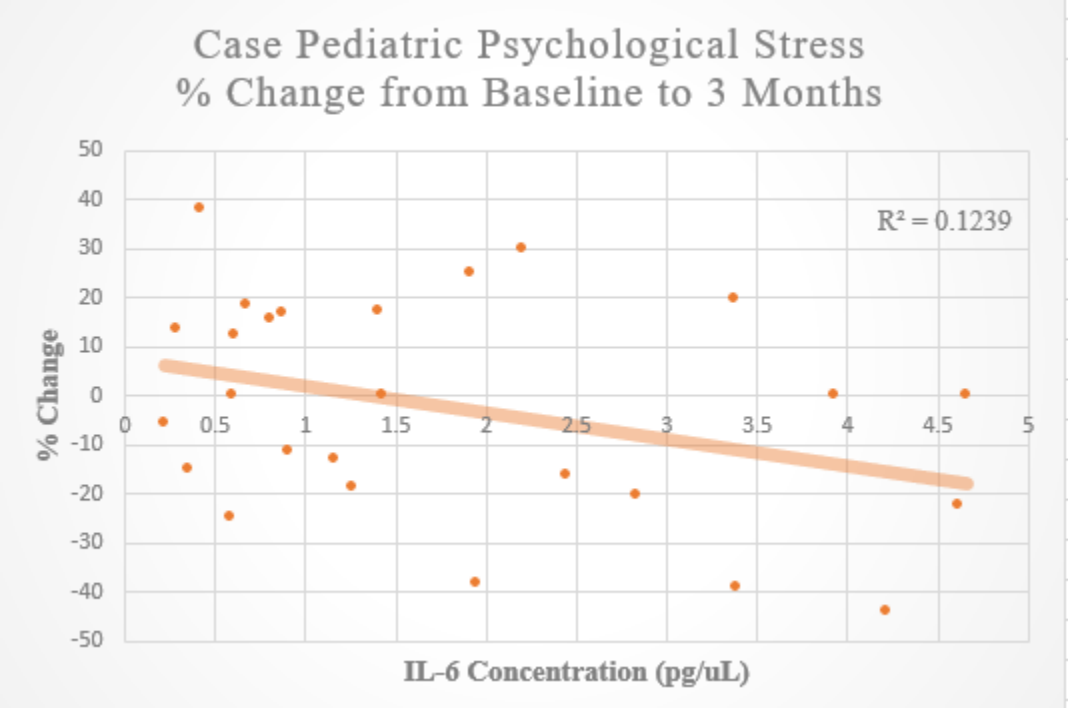

Figure 1. Case Pediatric Psychological Stress Percent Change from Baseline to 3 Months.

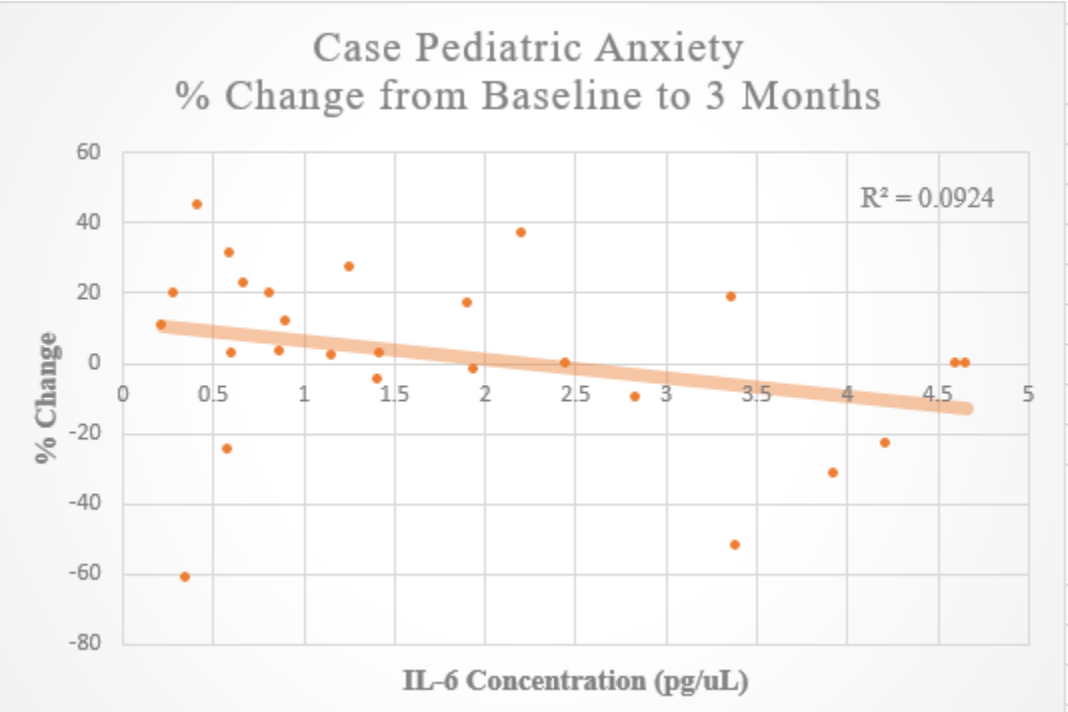

Figure 2. Case Pediatric Anxiety Percent Change from Baseline to 3 Months.

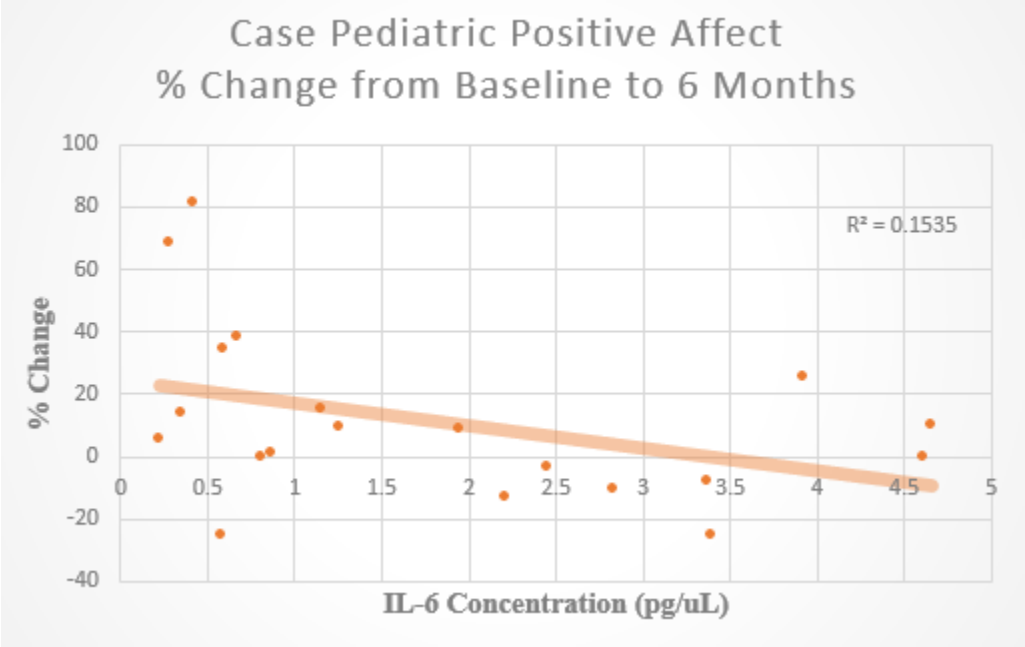

Figure 3. Case Pediatric Positive Affect Percent Change from Baseline to 3 Month.

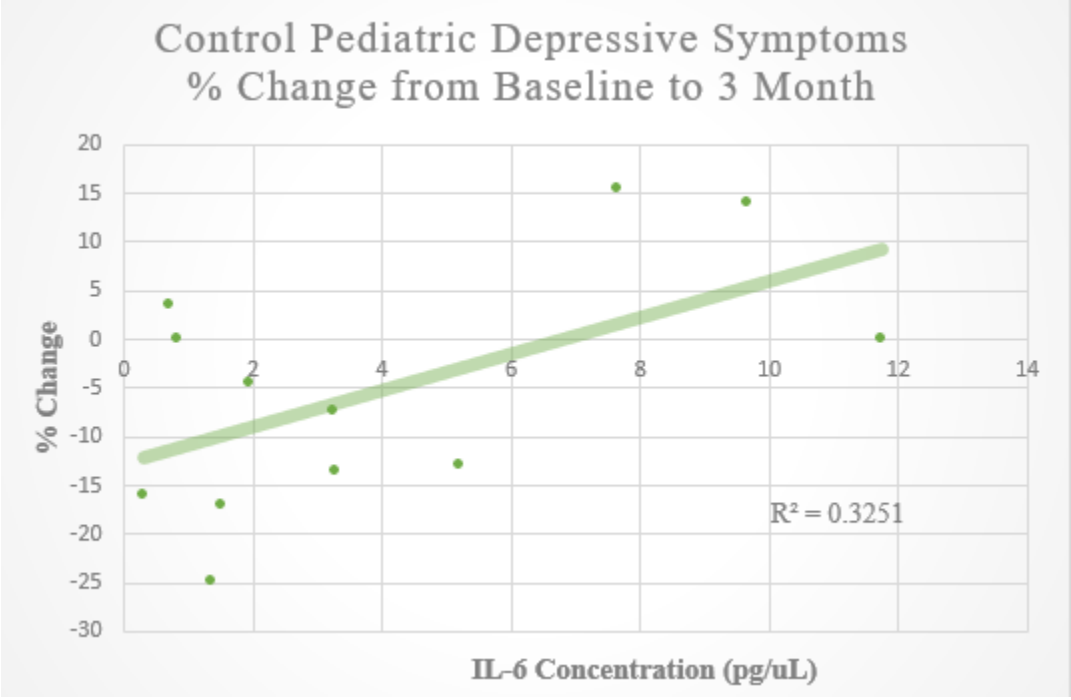

Figure 4. Control Pediatric Depressive Symptoms Percent Change from Baseline to 3 Month

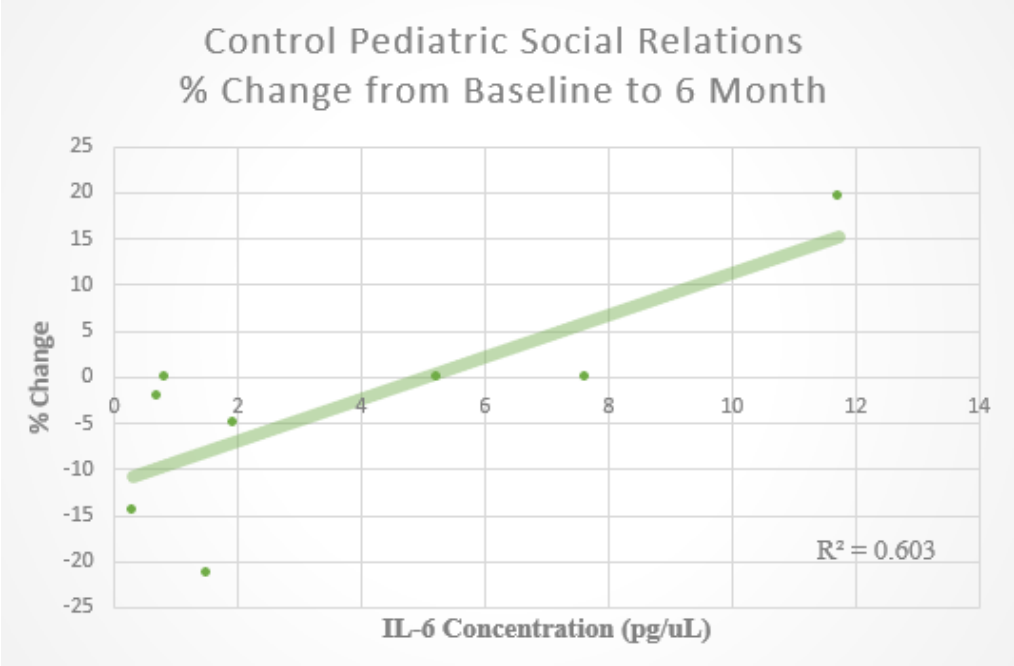

Figure 5. Control Pediatric Social Relations Percent Change from Baseline to 3 Month.

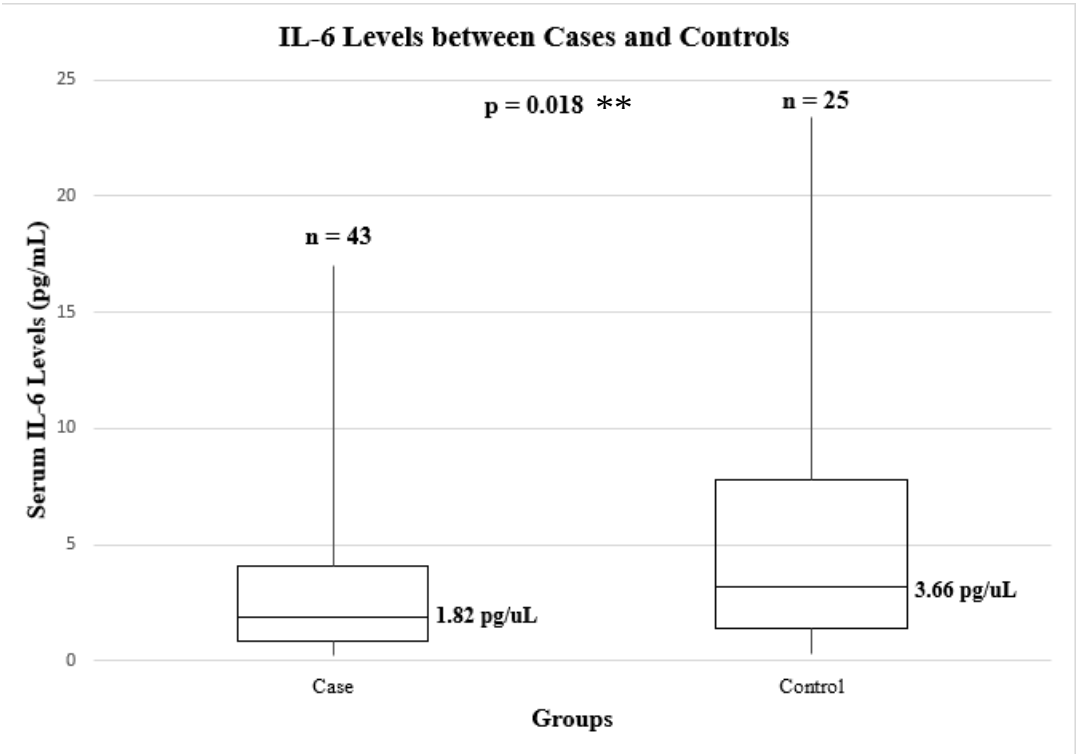

Figure 6. IL-6 Levels between Cases and Controls. Statistical significance is denoted (*) for *p*-values < 0.05.

	Parental Proxy					*Pediatric*							
	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Fatigue	Cognitive Function	Social Relations
% Change (mean ± SD)	3.36 (23.41)	-2.36 (25.97)	-2.97 (19.83)	0.55 (19.11)	2.82 (17.10)	2.32 (25.12)	-3.69 (22.17)	-2.37 (22.20)	2.54 (22.10)	-1.44 (12.56)	-1.93 (15.48)	0.91 (15.02)	-2.36 (14.36)
R^2	0.044	0.017	0.025	0.015	0.007	0.092	0.036	0.123	0.122	0.025	0.008	0.046	0.038
p-value	0.374	0.177	0.106	0.343	0.391	0.049*	0.109	0.173	0.433	0.106	0.116	0.382	0.076

Table 3: Case Baseline to 3 Months Percent Change in Outcome (n = 26). Significant *p*-values below <0.05 are marked with an (*).

	Parental Proxy					*Pediatric*							
	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Fatigue	Cognitive Function	Social Relations
% Change (mean ± SD)	-1.94 (23.20)	-1.04 (24.75)	0.32 (23.72)	-3.33 (19.27)	5.64 (18.08)	-3.37 (29.07)	-2.79 (20.32)	0.32 (23.72)	11.25 (27.56)	-1.14 (19.61)	0.07 (19.79)	0.10 (17.65)	-1.73 (16.97)
R^2	0.003	0.003	0.001	0.05	0.026	0.046	0.038	0.034	0.153	0.133	0.007	0.024	0.073
p-value	0.238	0.304	0.165	0.124	0.179	0.217	0.161	0.389	0.021*	0.389	0.348	0.338	0.181

Table 4: Case Baseline to 6 Months Percent Change in Outcome (n = 20). Significant *p*-values below <0.05 are marked with an (*).

	Parental Proxy					*Pediatric*							
	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Fatigue	Cognitive Function	Social Relations
% Change (mean ± SD)	0.12 (14.60)	-5.65 (15.28)	-0.73 (12.49)	-1.91 (16.44)	-6.74 (10.16)	0.19 (14.33)	-5.29 (12.43)	-7.94 (12.11)	-2.48 (13.64)	0.80 (13.39)	-3.20 (20.04)	4.35 (12.92)	-10.73 (9.17)
R^2	0.001	0.046	0.053	0.082	0.002	0.086	0.325	0.051	0.043	0.080	0.045	0.047	0.026
p-value	0.197	0.028*	0.117	0.125	0.002**	0.197	0.014*	0.003**	0.069	0.223	0.123	0.117	< 0.0001*

Table 5: Control Baseline to 3 Months Percent Change in Outcome (n = 12). Significant p-values below <0.05 are marked with an (*).

	Parental Proxy					*Pediatric*							
	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Anxiety	Depressive Symptoms	Psych Stress	Positive Affect	Global Health	Fatigue	Cognitive Function	Social Relations
% Change (mean ± SD)	-13.58 (14.93)	-9.11 (22.15)	-0.87 (15.88)	5.48 (21.67)	-2.73 (8.02)	-1.53 (23.09)	5.23 (11.57)	-6.71 (9.88)	5.18 (26.44)	-4.30 (7.93)	-4.61 (11.25)	23.60 (14.87)	-2.60 (12.03)
R^2	0.328	0.075	0.461	0.020	0.007	0.072	0.006	0.028	0.154	0.003	0.085	0.072	0.603
p-value	0.012*	0.075	0.097	0.460	0.041*	0.263	0.499	0.007*	0.457	0.018*	0.112	0.002**	0.086

Table 6: Control Baseline to 6 Months Percent Change in Outcome (n = 8). Significant *p*-values below <0.05 are marked with an (*).

Pediatric Global Health 7+2

Please respond to each question or statement by marking one box per row.

		Excellent	Very Good	Good	Fair	Poor
Global01R1	In general, would you say your health is:.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global02R1	In general, would you say your quality of life is:.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global03R1	In general, how would you rate your physical health?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Global04R1	In general, how would you rate your mental health, including your mood and your ability to think?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

		Never	Rarely	Sometimes	Often	Always
PedGlobal2R1	How often do you feel really sad?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

		Always	Often	Sometimes	Rarely	Never
PedGlobal5R1	How often do you have fun with friends?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PedGlobal6R1	How often do your parents listen to your ideas?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

In the past 7 days…

		Never	Almost Never	Sometimes	Often	Almost Always
2876R1r	I got tired easily	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3793R1r	I had trouble sleeping when I had pain.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Positive Affect – Short Form 8a

Please respond to each question or statement by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
SWB_P_027R1	I felt happy.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_025R1	I felt great.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_026R1	I felt cheerful	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_029R1	I felt joyful.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_037R1	I was in a good mood.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_049R1	I felt refreshed.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_001R1	I felt calm.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_004R1	I felt peaceful	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Psychological Stress Experiences – Short Form 8a

Please respond to each question or statement by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
EOS_P_011R1	I felt stressed.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_064R1	I felt that my problems kept piling up	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_067R1	I felt overwhelmed.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_112R1	I felt unable to manage things in my life..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_048R1	Everything bothered me.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_063R1	I felt under pressure	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_105R1	I had trouble concentrating.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EOS_P_118R1	I felt I had too much going on	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Pediatric Anxiety – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	**Never**	**Almost Never**	**Sometimes**	**Often**	**Almost Always**
2220R2r	I felt like something awful might happen..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
713R1r	I felt nervous.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
227bR1r	I felt scared.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5044R1r	I felt worried.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3459bR1r	I worried when I was at home	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
2230R1r	I got scared really easy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
231R1r	I worried about what could happen to me ..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3150bR2r	I worried when I went to bed at night	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Pediatric Depressive Symptoms – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	**Never**	**Almost Never**	**Sometimes**	**Often**	**Almost Always**
488R1r	I could not stop feeling sad	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
461R1r	I felt alone.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5041R1r	I felt everything in my life went wrong.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
5035R1r	I felt like I couldn't do anything right.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
711R1r	I felt lonely	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
228R1r	I felt sad.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
712R1r	I felt unhappy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
3952aR2r	It was hard for me to have fun.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Pediatric Social Relations- Interaction with Peers – Short Form

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	Never	Almost never	Sometimes	Often	Almost always
NQSCLped11	I felt close to my friends.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped12	I was able to count on my friends.……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped20	I felt comfortable with others my age….. .	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped28	I was happy with the friends I had.	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped30	I felt comfortable talking with my friends……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped32	I spent time with my friends.……...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped36	My friends and I helped each other out……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQSCLped38	I had fun with my friends.……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Pediatric Cognitive Function – Short Form

Please respond to each question or statement by marking one box per row.

		Not at all	A little bit	Somewhat	Quite a bit	Very much
NQCOGped03	I forget schoolwork that I need to do…...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped05	I sometimes forget what I was going to say……...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped08	I react slower than most people my age when I play games.	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped15	I forget things easily.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped17	I have trouble remembering to do things (e.g., school projects)……..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped18	It is hard for me to concentrate in school……..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped19	I have trouble paying attention to the teacher…….	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
NQCOGped20	I have to work really hard to pay attention or I will make a mistake……...	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

Pediatric Fatigue –Short Form

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	None of the time	A little bit of time	Some of the time	Most of the time	All of the time
NQFTGped01	I felt tired……...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped04	I had trouble starting things because I was too tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped05	I had trouble finishing things because I was too tired……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped06	I needed to sleep during the day……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped08	Being tired made it hard to play or go out with my friends as much as I would like...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped11r1	I was too tired to eat……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped12	Being tired makes me sad……..	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
NQFTGped13	Being tired makes me mad……	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Physical Stress Experiences – Short Form 8a

Please respond to each question or statement by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
EoS_S_032_PXR1	My child's heart beat faster than usual, even when he/she was not exercising or playing hard	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_033_PXR1	My child had trouble breathing, even when he/she was not exercising or playing hard	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_039_PXR1	My child's body shook	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_046_PXR1	My child had pain that really bothered him/her.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_017_PXR1	My child's muscles felt tight	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_024_PXR1	My child's mouth was dry	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_042_PXR1	My child had a headache	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_S_044_PXR1	My child's back hurt.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Positive Affect – Short Form 8a

Please respond to each question or statement by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
SWB_P_027_PXR1	My child felt happy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_025_PXR1	My child felt great	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_026_PXR1	My child felt cheerful	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_029_PXR1	My child felt joyful.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_037_PXR1	My child was in a good mood	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_049_PXR1	My child felt refreshed	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_001_PXR1	My child felt calm	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SWB_P_004_PXR1	My child felt peaceful.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Psychological Stress Experiences – Short Form 8a

Please respond to each question or statement by marking one box per row.

In the past 7 days…

		Never	Rarely	Sometimes	Often	Always
EoS_P_011_PXR1	My child felt stressed.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_064_PXR1	My child felt that his/her problems kept piling up.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_067_PXR1	My child felt overwhelmed.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_112_PXR1	My child felt unable to manage things in his/her life.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_048_PXR1	Everything bothered my child	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_063_PXR1	My child felt under pressure.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_105_PXR1	My child had trouble concentrating.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
EoS_P_118_PXR1	My child felt he/she had too much going on.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Parent Proxy Anxiety – Short Form 8a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	**Never**	**Almost Never**	**Sometimes**	**Often**	**Almost Always**
Pf1anxiety8r	My child felt nervous.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2anxiety2r	My child felt scared.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2anxiety9r	My child felt worried.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2anxiety1r	My child felt like something awful might happen.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2anxiety5r	My child worried when he/she was at home	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf1anxiety1r	My child got scared really easy	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf1anxiety3r	My child worried about what could happen to him/her	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2anxiety4r	My child worried when he/she went to bed at night	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Parent Proxy Depressive Symptoms – Short Form 6a

Please respond to each question or statement by marking one box per row.

	In the past 7 days…	**Never**	**Almost Never**	**Sometimes**	**Often**	**Almost Always**
Pf2depr7r	My child could not stop feeling sad	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf1depr7r	My child felt everything in his/her life went wrong.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf1depr5r	My child felt like he/she couldn't do anything right	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2depr10r	My child felt lonely.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2depr3r	My child felt sad.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Pf2depr6r	It was hard for my child to have fun	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

